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# Chaetomium-like fungi causing opportunistic infections in humans: a possible role for extremotolerance

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**Abstract** Members of the family *Chaetomiaceae* are ubiquitous ascosporulating fungi commonly, which reside in soil enriched with manure or cellulosic materials. Their role as human pathogens is largely ignored. However, the ability of some species to grow at high temperature enables them to play an important role as opportunistic pathogens. The family contains several genera and species that have never been reported to cause human infection. Hereby, three new species are described; two belong to the genus *Subramaniula* and one represents a *Chaetomium* species. *Subramaniula asteroides* was isolated from various sources including eye and skin infections as well as from the natural environment, and *S. obscura* was isolated from a toe infection. *Chaetomium anamorphosum* was isolated from a kidney transplant patient suffering from fungal peritonitis. All species described were previously misidentified as *Papulaspora* spp. due to the

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formation of cellular clumps or bulbil-like structures, which are characteristic of *Papulaspora*. The isolates failed to form sexual fruit bodies and ascospores remained absent, which is an unusual feature for the generally ascosporulating genera *Chaetomium* and *Subramaniula*; minute conidia from phialides were sometimes observed.

**Keywords** *Chaetomium* · Desert fungi · Keratitis · *Papulaspora* · Peritonitis · Sterile fungi · *Subramaniula*

## Introduction

Melanized fungi are important causes of human infection. About 70 genera representing hundreds of species have been implicated in human disease (de Hoog et al. 2013a; Guppy et al. 1998; Revankar and Sutton 2010; Revankar et al. 2002). Several main ecological groups can be distinguished. Members of *Chaetothyriales* exhibit pronounced virulence and cause deep and systemic infections in immunocompetent humans, e.g. chromoblastomycosis or brain infection (Badali et al. 2009; Revankar and Sutton 2010). Members of *Pleosporales* are preponderantly found as degraders of plants debris or as mild opportunistic pathogens; human infections mostly comprise traumatic inoculation of contaminated materials (Revankar and Sutton 2010). Recently, the significance of *Sordariales* was underlined (Badali et al. 2011; de Hoog et al. 2013b), particularly the *Chaetomiaceae* whose prevalence has been underestimated because of diagnostic problems.

Phenotypically, the identification of clinical chaetomium-like fungi has been difficult as a large proportion of them fail to produce typical diagnostic structures in culture (Najafzadeh et al. 2014; Vinod Mootha et al. 2012). Until recently, fungi lacking propagation in the form of conidia were treated in the clinical laboratory as unidentifiable '*mycelia sterilia*' (Pounder et al. 2007; Santos et al. 2013; Vinod Mootha et al. 2012). Isolates forming clumps or bulbils were referred to as *Papulaspora*, whereas filamentous strains from subcutaneous infection were known as *Madurella* (de Hoog et al. 2013b). With the application of molecular phylogenetic multi-gene DNA sequence analyses, such isolates were found to be of high diversity being distributed to many genera and families of ascomycetes. In addition, species proved to comprise several sibling species that were previously thought to represent a single taxon. For example, *Madurella* is now known to harbour four species (de Hoog et al. 2012).

Despite recent progress in the taxonomy of sterile fungi, the phylogenetic position of *Papulaspora* remains uncertain. The occurrence of bulbils or papulospores is non-specific and polyphyletic, as similar compact masses of cells can be produced by a wide range of fungi from different genera and orders (Hotson 1917). The generic type species *Papulaspora sepedonioides* is a member of the order *Melanosporales* (Davey et al. 2008), but further papulaspora-like asexual morphs have been assigned to *Ceratostoma* and *Chaetomium* in the order *Sordariales* (Bainier 1907; Hotson 1917; Weresub and LeClair 1971; Zang et al. 2004). Isolates previously misidentified as *P. sepedonioides* are currently listed as non-sporulating *Chaetomium* species (Vinod Mootha et al. 2012).

Recently, more attention has been paid to the non-sporulating chaetomium-like isolates that cause human infection (Najafzadeh et al. 2014; Vinod Mootha et al. 2012), such as keratitis or subcutaneous infection after trauma. Despite application of molecular phylogenetic methods, researchers were unable to identify these isolates down to species level in the genus *Chaetomium* because of the present state of morphological confusion and high phylogenetic divergence in the genus. *Chaetomium* contains more than 300 described species, but modern descriptions of most taxa are lacking, and very few have been circumscribed with DNA data. In the present concept of the genus, species that produce elaborate sporocarps are accepted, and most species lack asexual morphs (von Arx et al. 1986). Species are generally cosmopolitan and reside in soil on cellulose-rich materials or on dung (Bell 2005; Carter and Khan 1982; Doveri 2008; von Arx et al. 1986). A certain prevalence of chaetomium-like species was noted in desert soil subjected to conditions of dryness and extremely variable temperatures (Rodríguez et al. 2004). Members of the madurella-clade phylogenetically located inside the genus *Chaetomium* are typically confined to areas with arid climates. *Madurella* species are consistent agents of human subcutaneous mycoses, and the arid areas of northeastern Africa are endemic for human mycetoma (Ahmed et al. 2002). Most human infections by chaetomium-like species concern traumatic inoculations into otherwise healthy humans, and rarely occur as deep infections in severely immunocompromised hosts (Al-Aidaroos et al. 2007; Guppy et al. 1998; Hubka et al. 2011).

In the present paper we describe three novel species from clinical sources that were earlier provisionally identified as *Papulaspora* or *Chaetomium* species. One of the species was represented by seven clinical isolates particularly from the Middle East, while an environmental strain was derived from hydrocarbon-polluted desert soil. This raises the question whether in these fungi there is a connection between growth under arid conditions and ability to cause opportunistic infections.

## Materials and methods

### Isolates and morphology

Strains studied were isolated from human patients, or from hydrocarbon-rich desert soil using enrichment under toluene

atmosphere according to Zhao et al. (2010). Reference strains were obtained from the CBS reference collection (Table 1). Morphology and colony characteristics were examined on Malt Extract Agar (MEA, Oxoid, U.K.) and Oatmeal Agar (OA, home-made at CBS) and incubated for 2 weeks at 25 °C in the dark. Microscopic features were examined on OA, MEA and tap water agar slide cultures. To induce sporulation, isolates were incubated under UV light in a 12-h light/dark regimen and examined every 7 d for up to 8 weeks. Mounted slides were examined with a Nikon ECLIPSE 80i microscope and photographs were captured using a Nikon digital sight DS-5M camera attached to the microscope. Colony growth rates were determined on MEA plates incubated for 1 week at temperatures ranging from 6 to 36 °C at 3 °C intervals including 37 and 40 °C.

### DNA extraction, amplification and sequencing

Genomic DNA was extracted using a cetyltrimethyl ammonium bromide (CTAB) method described previously by Möller et al. (1992). Amplification and sequencing were performed for the Internal Transcribed Spacer (ITS) and D1/D2 domains of the 28S rRNA gene, partial translation elongation factor 1-α (*TEF1*), β-tubulin (*Btub*), DNA-dependent RNA polymerase II largest subunit (*RPB1*) and second largest subunit (*RPB2*). Primers used for amplification and sequencing are according to de Hoog et al. (2013a).

### Alignment and phylogenetic analyses

DNA sequences were assembled and edited using SEQMAN from the Lasergene package (DNASTAR, Madison, WI, U.S.A.). Sequences were deposited in GenBank; accession numbers are listed in Table 1. Two alignments were generated to study the phylogenetic position of the unknown species. Sequences were aligned with the online version of MAFFT v. 7 (http://mafft.cbrc.jp) and manually adjusted using BIOEDIT v. 7.1.3 software (Hall 1999). Each gene was aligned independently and concatenated matrices were prepared using DATACONVERT v. 1.0. The first alignment consisted of ribosomal ITS and LSU sequences of representative species of *Chaetomium*, *Chaetomidium*, *Thielavia*, *Papulaspora*, *Subramaniula*, and *Madurella*. The second alignment consisted of the protein coding loci *TEF1*, *Btub*, *RPB1* and *RPB2* sequences of a selected number of strains. Alignments and trees were deposited in TreeBASE database (TreeBASE ID: 17309).

Phylogenetic analyses using Maximum likelihood were performed in RAxML v. 8.0.24 (Stamatakis 2014). Bayesian analyses with default priori of MRBAYES v. 3.1.2 were conducted using the CIPRES Science Gateway server. Two simultaneous Markov chain Monte Carlo samplings were performed with four chains of which one was cold and three were heated. The run was conducted for 30,000,000 generations with sampling every 100 generations and the 'burn in' was set at 25 % of resulting trees. Convergence was evaluated from the two independent runs using AWTY and TRACER v. 1.5 (Nylander et al. 2008; Rambaut and Drummond 2007).

## Results

Phylogenetic analyses of the combined ITS and LSU loci were used to establish the phylogenetic position of the three unknown species. The analyses included 87 sequences with in total 1384 characters, including alignment gaps. In general, the genera of *Chaetomiaceae* (*Chaetomidium*, *Chaetomium*, *Subramaniula*, *Thielavia* and the asexual genera *Madurella* and *Papulaspora*) could not be resolved (Fig. 1). *Chaetomium*, *Papulaspora* and *Thielavia* were polyphyletic and their taxonomy requires further investigation to define their generic boundaries. *Subramaniula* and *Madurella* formed monophyletic (sub)clades within *Chaetomium*. The ITS and LSU data provided insufficient resolution to ascertain species delimitations. The unidentified isolates clustered in a single, supported clade [Maximum likelihood bootstrap support values (ML-BS) / posterior probabilities (PP) 97 %/0.94] in the basal lineages of the *Chaetomiaceae*. The clade contained *Subramaniula thielavioides*, which is the type species of *Subramaniula* and could therefore be regarded as the *Subramaniula* clade. The clade was phylogenetically distant from *Papulaspora equi*, represented by the ex-type strain (CBS 573.89), and from *P. sepedonioides* (strain CBS 265.79), as well as from all described *Madurella* species. No match was found with any other *Chaetomium*, *Thielavia* or related species.

A multi-gene phylogeny with *Btub*, *TEF1*, *RPB1* and *RPB2* was used focusing on the *Subramaniula* clade (Fig. 2). The analysis included 20 taxa with a total of 3571 alignment characters. Three new species were identified, of which two were found to be closely related to *Subramaniula thielavioides* (ML-BS/PP, 100 %/1.0; Fig. 2) nested within *Chaetomium*, and were considered to belong to *Subramaniula*. The proposed species *S. obscura* was a sister to the type species of *Subramaniula*, *S. thielavioides*, in a well-supported subclade (ML-BS/ PP, 99 %/1.0), whereas *S. asteroides* formed a subclade sister to *S. thielavioides* and *S. obscura*.

With the four partial protein coding loci, the subcluster of *S. asteroides* was split into three supported lineages (Fig. 2). The first lineage (ML-BS/PP, 95 %/1.0) contained four strains, three of which were of clinical origin and one environmental strain that had been selectively isolated by toluene enrichment from desert soil in Saudi Arabia. The second lineage (ML-BS/PP, 100 %/1.0) contained three isolates from cases of keratitis

**Table 1** Strains information and GenBank accession numbers of sequences (sequences of strains with five genes listed were generated in this study)

| Name | Isolate No. | Alternative number | Provisional identification and type [T] | Source | ITS | LSU | *Btub* | *rPB1* | *rPB2* | *TEF1* |
|---|---|---|---|---|---|---|---|---|---|---|
| *Chaetomidium arxii* | CBS 104.79 | | [T] *Chaetomidium arxii* | Dung | JX280770 | FJ666359 | – | – | – | – |
| *Chaetomidium leptoderma* | CBS 113678 | | [T] *Chaetomidium galaicum* | Soil | JN573175 | FJ666361 | – | – | – | – |
| *Chaetomidium leptoderma* | CBS 538.74 | | [T] *Thielavia leptoderma* | Soil | AF096171 | AF096186 | – | – | – | – |
| *Chaetomium acropullum* | CBS 114580 | | [T] *Chaetomium acropullum* | Soil | JX280763 | JX280662 | – | – | – | – |
| *Chaetomium amesii* | CBS 338.68 | VKM F-1948 | [T] *Chaetomium amesii* | | KP970640 | KP970663 | – | – | – | – |
| *Chaetomium anamorphosum* | CBS 137114 | dH24066, Kw207/12 | *Papulospora* sp. | Peritonitis | KP862598 | KP970641 | KP900704 | KP980684 | KP900667 | KP900687 |
| *Chaetomium apiculatum* | CBS 472.63 | | [T] *Chaetomium apiculatum* | Dung of monkey | KP970633 | KP970656 | – | – | – | – |
| *Chaetomium atrobrunneum* | CBS 128474 | UTHSC 09-101 | | Right lung | JX280776 | JX280671 | – | – | – | – |
| *Chaetomium atrobrunneum* | CBS 379.66 | | [T] *Chaetomium atrobrunneum* | Mouldy mattress | JX280771 | JX280666 | – | – | – | – |
| *Chaetomium atrobrunneum* | CBS 128459 | UTHSC 01-329 | | Brain | JX280773 | JX280668 | – | – | – | – |
| *Chaetomium bostrychodes* | CBS 188.63 | | | Sputum | JX280779 | JX280673 | – | – | – | – |
| *Chaetomium brasiliense* | CBS 761.83 | | | Soil | X280780 | JX280674 | – | – | – | – |
| *Chaetomium cuniculorum* | CBS 156.52 | | [T] *Chaetomium cristatum* | | KP862603 | KP970643 | KP900694 | KP980676 | KP900655 | KP900674 |
| *Chaetomium cuniculorum* | CBS 121.57 | | | | KP862602 | KP970644 | KP900709 | KP980687 | KP900671 | KP900690 |
| *Chaetomium erectum* | CBS 111.63 | | | Unknown | JX280782 | JX280676 | – | – | – | – |
| *Chaetomium erectum* | CBS 140.56 | | [T] *Chaetomium erectum* | Plant | HM449044 | HM449058 | – | – | – | – |
| *Chaetomium funicola* | CBS 139.56 | | [T] *Chaetomium causiiforme* | Cloths | JX280784 | JX280678 | – | – | – | – |
| *Chaetomium funicola* | CBS 179.84 | | [T] *Chaetomium variostiolatum* | Tarpaulin | JX280785 | JX280679 | – | – | – | – |
| *Chaetomium funicola* | CBS 154.52 | | | Unknown | JX280783 | JX280677 | – | – | – | – |
| *Chaetomium fuscum* | CBS 128480 | UTHSC 04-987 | | Knee | JX280789 | JX280681 | – | – | – | – |
| *Chaetomium fuscum* | CBS 140.50 | | [T] *Chaetomium fuscum* | Moist jute cloth | JX280787 | AF286396 | – | – | – | – |
| *Chaetomium fuscum* | CBS 128460 | UTHSC 03-1854 | | Pleural fluid | JX280788 | JX280680 | – | – | – | – |
| *Chaetomium fusisporum* | CBS 199.84 | TRTC 48900 | | | KP862601 | KP970645 | KP900707 | KP980685 | KP900653 | KP900691 |
| *Chaetomium globosporum* | CBS 108.83 | | [T] *Chaetomium globosporum* | Plant | JX280820 | JX280708 | – | – | – | – |
| *Chaetomium globosum* | CBS 128452 | UTHSC 08-954 | | Blood | JX280813 | JX280702 | – | – | – | – |
| *Chaetomium globosum* | CBS 128453 | UTHSC 03-1750 | | Scalp | JX280805 | JX280695 | – | – | – | – |
| *Chaetomium globosum* | CBS 148.51 | | | Plant | GU563374 | JX280684 | – | – | – | – |
| *Chaetomium globosum* | CBS 128443 | UTHSC 03-824 | | Nail | JX280798 | JX280693 | – | – | – | – |
| *Chaetomium globosum* | CBS 128477 | UTHSC 10-814 | | Skin | JX280797 | JX280692 | – | – | – | – |
| *Chaetomium globosum* | CBS 147.51 | | | Human | JX280799 | JX280707 | – | – | – | – |
| *Chaetomium hispanicum* | CBS 639.83 | BP 1975 | | Soil | KP970637 | KP970660 | – | – | – | – |
| *Chaetomium homopilatum* | CBS 520.80 | | | Dung | JX280826 | JX280713 | – | – | – | – |
| *Chaetomium homopilatum* | CBS 473.63 | | [T] *Chaetomium biapiculatum* | Dung | KP862605 | KP970642 | – | – | – | – |
| *Chaetomium homopilatum* | CBS 731.71 | | | Dung | JX280825 | JX280712 | – | – | – | – |
| *Chaetomium irregulare* | CBS 227.82 | | | | KP862599 | KP970646 | KP900705 | KP980691 | KP900668 | KP900688 |
| *Chaetomium irregulare* | CBS 446.66 | IMI 153340 | [T] *Chaetomium irregular* | | KP862600 | KP970647 | KP900706 | KP980692 | KP900669 | KP900689 |

**Table 1** (continued)

| Name | Isolate No. | Alternative number | Provisional identification and type [T] | Source | ITS | LSU | *Btub* | *rPB1* | *rPB2* | *TEF1* |
|---|---|---|---|---|---|---|---|---|---|---|
| *Chaetomium lucknowense* | CBS 796.71 | | | Soil | JX280827 | JX280714 | – | – | – | – |
| *Chaetomium madrasense* | CBS 366.83 | | | Dung | JX280791 | JX280682 | – | – | – | – |
| *Chaetomium madrasense* | CBS 110.83 | | [T] *Chaetomium gibberosporum* | Unknown | JX280790 | JX280683 | – | – | – | – |
| *Chaetomium mareoticum* | CBS 802.83 | | | Dung | JX280828 | JX280715 | – | – | – | – |
| *Chaetomium medusarum* | CBS 162.73 | | | Dung | JX280830 | JX280717 | – | – | – | – |
| *Chaetomium medusarum* | CBS 729.71 | | | Dung of deer | KP970635 | KP970658 | – | – | – | – |
| *Chaetomium medusarum* | CBS 148.67 | | [T] *Chaetomium medusarum* | Soil | JX280829 | JX280716 | – | – | – | – |
| *Chaetomium megalocarpum* | CBS 778.71 | ETH 1924 | [T] *Chaetomium megalocarpum* | Soil | KC109747 | KC109747 | – | – | – | – |
| *Chaetomium murorum* | CBS 566.85 | | | Soil | JX280832 | JX280719 | – | – | – | – |
| *Chaetomium murorum* | CBS 138.58 | | | Plant | JX280831 | JX280718 | – | – | – | – |
| *Chaetomium nigricolor* | CBS 317.74 | | [T] *Chaetomium verrucichaeta* | Soil | JX280836 | JX280720 | – | – | – | – |
| *Chaetomium nigricolor* | CBS 128455 | UTHSC 09-1153 | | Trachae | JX280835 | JX280723 | – | – | – | – |
| *Chaetomium nigricolor* | CBS 128478 | UTHSC 08-2214 | | Biopsy | JX280834 | JX280722 | – | – | – | – |
| *Chaetomium nozdrenkoae* | CBS 163.62 | ATCC 14528;IMI 090490;IMI 090490ii; MUCL 18703;VKM F-1953 | [T] *Chaetomium nozdrenkoae* | Soil | KP970636 | KP970659 | – | – | – | – |
| *Chaetomium pachypodioides* | CBS 164.52 | | [T] *Chaetomium pachypodioides* | Plant | GQ922526 | JX280727 | – | – | – | – |
| *Chaetomium pachypodioides* | CBS 128.85 | | [T] *Chaetomium intricatum* | Air | JX280839 | JX280726 | – | – | – | – |
| *Chaetomium perlucidum* | CBS 141.58 | | [T] *Chaetomium perlucidum* | Plant | JX280840 | JX280728 | – | – | – | – |
| *Chaetomium seminis-citrulli* | CBS 143.58 | | [T] *Chaetomium semenis-citrulli* | Dung | JX280841 | JX280729 | – | – | – | – |
| *Chaetomium subglobosum* | CBS 149.60 | | [T] *Chaetomium subglobosum* | Plant | JX280852 | HM751083 | – | – | – | – |
| *Chaetomium subspirilliferum* | CBS 150.60 | | [T] *Chaetomium subspirilliferum* | Soil | JX280853 | JX280735 | – | – | – | – |
| *Chaetomium subspirilliferum* | CBS 194.84 | | [T] *Chaetomium barilochense* | Dung | KP970639 | KP970662 | – | – | – | – |
| *Chaetomium uniporum* | CBS 109.83 | ETH 7503 | [T] *Chaetomium uniporum* | Soil | KP970634 | KP970657 | – | – | – | – |
| *Chaetomium vitellinum* | CBS 250.85 | | [T] *Achaetomium thermophilum* | Plant | JX280859 | JX280740 | – | – | – | – |
| *Madurella fahalii* | CBS 129176 | | [T] *Madurella fahalii* | Mycetoma | JN573178 | JX280751 | – | – | – | – |
| *Madurella mycetomatis* | CBS 110359 | | | Mycetoma | JX280861 | JX280746 | – | – | – | – |
| *Madurella mycetomatis* | CBS 132259 | | | Mycetoma | JX280866 | JX280749 | – | – | – | – |
| *Madurella mycetomatis* | CBS 109801 | | [T] *Madurella mycetomatis* | Mycetoma | DQ836767 | JX280743 | – | – | – | – |
| *Madurella mycetomatis* | CBS 132419 | | | Mycetoma | JX280864 | JX280748 | – | – | – | – |
| *Madurella pseudomycetomatis* | CBS 129177 | | [T] *Madurella mycetomatis* | Mycetoma | EU815933 | JX280752 | – | – | – | – |
| *Madurella pseudomycetomatis* | CBS 248.48 | | | Mycetoma | JX280868 | JX280753 | – | – | – | – |
| *Madurella tropicana* | CBS 201.38 | | | Mycetoma | JX280869 | JX280750 | – | – | – | – |
| *Papulaspora equi* | CBS 128687 | | | Buttock | JX280872 | JX280757 | KT006927 | KP970664 | KP900660 | KP900679 |
| *Papulaspora equi* | CBS 573.89 | | [T] *Papulaspora equi* | Ocular lesion | JX280870 | JX280755 | KT006928 | KP970665 | KP900663 | KP900692 |
| *Papulaspora equi* | CBS 128684 | | | Eye | JX280871 | JX280756 | – | – | – | – |

**Table 1** (continued)

| Name | Isolate No. | Alternative number | Provisional identification and type [T] | Source | ITS | LSU | *Btub* | *rPB1* | *rPB2* | *TEF1* |
|---|---|---|---|---|---|---|---|---|---|---|
| *Papulaspora sepedonioides* | CBS 265.79 | | | Soil | KP970638 | KP970661 | KP900693 | KP994920 | KP900654 | KP900673 |
| *Subramaniula asteroides* | CBS 128466 | UTHSC 07-434; dH 21617 | *Papulospora* sp. | Corneal ulcer | JX280843 | JX280732 | KP900695 | KP980677 | KP900656 | KP900675 |
| *Subramaniula asteroides* | CBS 128679 | UTHSC 01-976; dH 21643 | *Papulospora* sp. | | KP862591 | KP970648 | KP900696 | KP980678 | KP900657 | KP900676 |
| *Subramaniula asteroides* | CBS 123294 | UTHSC 03-1576; dH 13222 | *Papulospora* sp. | Corneal ulcer | HQ906667 | JX280731 | KP900703 | KP980690 | KP900666 | KP900686 |
| *Subramaniula asteroides* | CBS 128689 | UTHSC 08-126; dH 21654 | *Papulospora* sp. | | KP862592 | KP970649 | KP900699 | KP980680 | KP900661 | KP900680 |
| *Subramaniula asteroides* | CBS 128685 | UTHSC 05-2439; dH 21650 | *Papulospora* sp. | | KP862593 | KP970650 | KP900698 | KP980679 | KP900659 | KP900678 |
| *Subramaniula asteroides* | CBS 128681 | UTHSC 03-1576; dH 21645 | *Papulospora* sp. | Corneal ulcer | KP970631 | KP970651 | KP900697 | KP980689 | KP900658 | KP900677 |
| *Subramaniula asteroides* | dH 21571 | Fungiscope ID 1532908 | *Papulospora* sp. | Keratitis | JX280842 | JX280730 | KP900701 | KP980682 | KP900664 | KP900682 |
| *Subramaniula asteroides* | CBS 138726 | SA 38 | *Papulospora* sp. | Polluted soil | KP862594 | KP970652 | KP900702 | KP980683 | KP900665 | KP900683 |
| *Subramaniula asteroides* | | UTHSC 03-1315 | *Papulospora* sp. | Skin biopsy | KP970632 | | | | | |
| *Subramaniula asteroides* | | CNM-CM 4314 | | Corneal exudate | KP970629 | | | | | |
| *Subramaniula asteroides* | | CNM-CM 7482 | | Endophthalmitis | KP970630 | | | | | |
| *Subramaniula obscura* | CBS 132916 | dH 22373, MF1041/11 | | Toe infection | KP862595 | KP970653 | KP900700 | KP980681 | KP900662 | KP900681 |
| *Subramaniula thielavioides* | CBS 122.78 | | [T] *Achaetomium thielavioides* | Dung | KP862597 | KP970654 | KP900708 | KP980686 | KP900670 | KP900685 |
| *Subramaniula thielavioides* | CBS 560.84 | | | | KP862596 | KP970655 | KP900710 | KP980688 | KP900672 | KP900684 |
| *Thielavia fragilis* | CBS 456.73 | | [T] *Chaetomidium fragile* | Soil | AJ271578 | JX280758 | – | – | – | – |
| *Thielavia hyalocarpa* | CBS 646.74 | | | Soil | AJ271583 | JX280759 | – | – | – | – |
| *Thielavia subthermophila* | CBS 509.74 | | [T] *Thielavia subthermophila* | Soil | JX280873 | JX280760 | – | – | – | – |
| *Thielavia subthermophila* | CBS 125981 | | | Brain | HM448441 | HM448442 | – | – | – | – |
| *Thielavia subthermophila* | AM909688 | | | Plant | AM909688 | AM909688 | – | – | – | – |

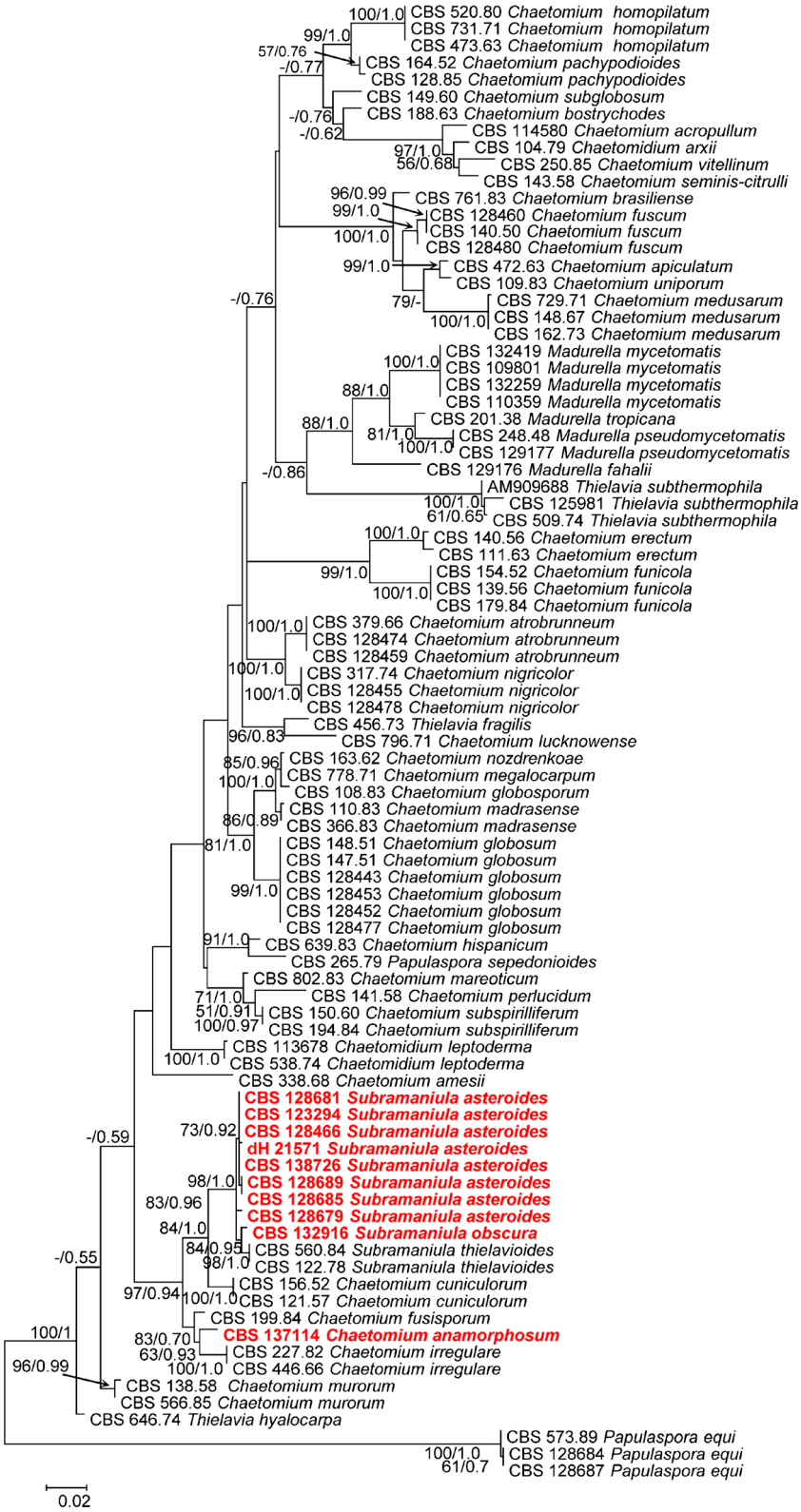


**Fig. 1** Phylogram of representative selection of *Chaetomiaceae* genera obtained by Maximum likelihood and Bayesian analysis of ITS and LSU. Maximum likelihood bootstrap (ML-BS) and Bayesian posterior probability (PP) are indicated at the nodes. *Papulaspora equi* was used to root the tree

from the U.S.A. The clinical strain from India, dH 21571= Fungiscope 1532908 deviated slightly from the type.

No match was found for our strains with any existing taxon on NCBI's GenBank nucleotide database. All of our strains are nested within *Chaetomium sensu lato* (Fig. 1), but distant from the generic type species, *C. globosum*. The new species proposed in this study cluster sister to strains of *Subramaniula thielavioides* (the type species of *Subramaniula*, nestled

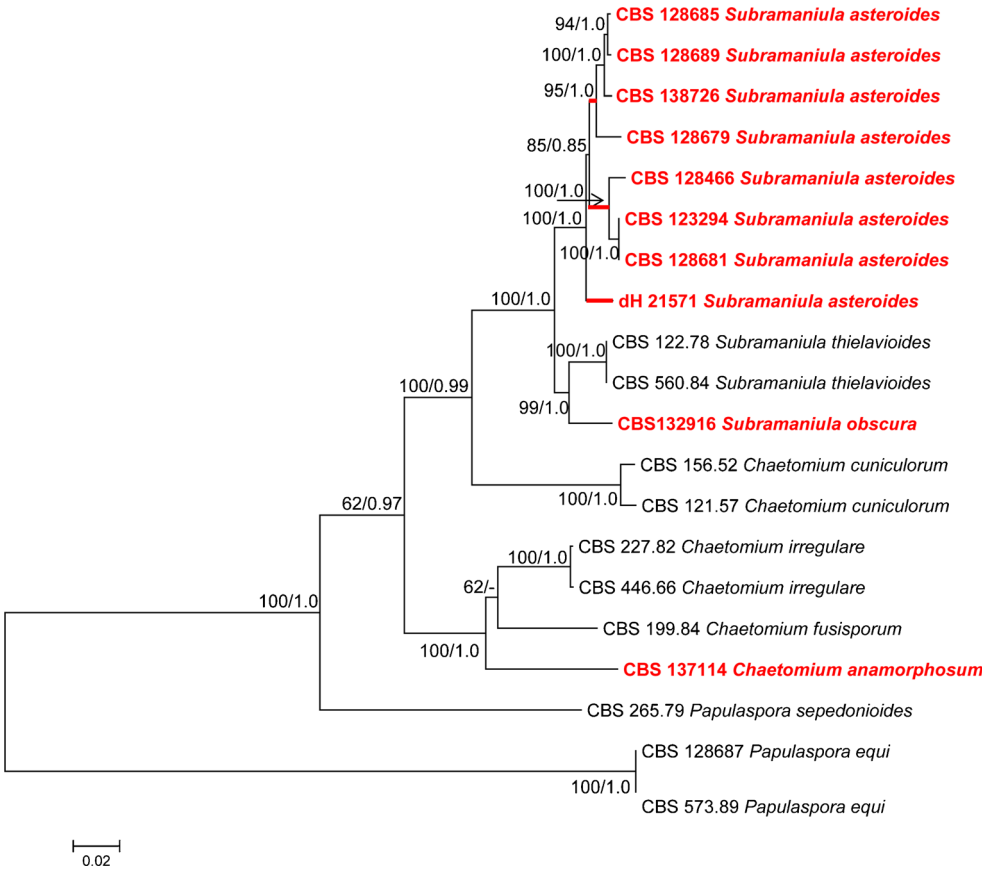


**Fig. 2** Phylogeny of combined data (*TEF1*, *rPB1*, *rPB2*, and *Btub*) of *Subramanuila* clade obtained by Bayesian and Maximum likelihood analysis. Maximum likelihood bootstrap (ML-BS) and Bayesian posterior probability (PP) are indicated at the nodes. *Papulaspora equi* was used as out group

within *Chaetomium sensu lato*) and are treated in this study as species of *Subramaniula*, pending a more comprehensive taxonomic study of the *Chaetomiaceae*. An isolate recovered from a case of peritonitis and originating from Kuwait was found to be closely related to two *Chaetomium* species, i.e. *C. irregulare* and *C. fusisporum* (ML-BS/PP, 100/1.0; Fig. 2); this strain was identified as a novel species, *C. anamorphosum* (Figs. 1 and 2).

## Taxonomy

***Chaetomium anamorphosum*** S.A. Ahmed, Z. U. Khan, X. Wang & de Hoog, **sp. nov.** – Fig. 3, MB 810426

Colonies on MEA velvety, white, becoming yellow to tan with age: reverse buff to yellow. Colonies on OA dark yellowish in the centre becoming faint to colourless toward the margin; mycelium immersed, with a yellow pigment diffusing into the agar. Hyphae hyaline, partially converting to dark brown or black, thick-walled chlamydospore-like structures. Cellular clumps seen on the surface of colonies as small, black, spherical to irregular structures; under the light microscopic they are black, consisting of aggregates of thick-walled cells, 5–10×4–6 μm. Phialides hyaline, erect, short cylindrical or broader at the base. Conidia smooth-walled, hyaline, unicellular, 2–3×1.5–2.0 μm, obovoidal or ellipsoidal. Minimum growth temperature 6 °C, optimum 33–36 °C, maximum above 40 °C (Fig. 4).

Holotype: dried culture in CBS Herbarium CBS H-21973; type culture CBS 137114, from case of peritonitis, Kuwait, Z.U. Khan.

## Case report of *Chaetomium anamorphosum* CBS 137114

A 21-year-old Jordanian patient with a history of end-stage renal disease underwent a kidney transplant in 1995. The transplanted kidney was rejected in 2007. The patient was put on peritoneal dialysis at regular intervals. In February 2011, the patient underwent radical nephrectomy of transplanted and native kidney because of uncontrolled hypertension. In April 2012, the patient presented with complaints of abdominal pain, vomiting and constipation. A CT scan of the abdomen revealed partial small bowel obstruction (ileus). Accompanying symptoms at this time were uncontrolled hypertension and seizures associated with posterior reversible encephalopathy syndrome seen in a CT scan of the brain. He was admitted to the Nephrology Unit of the Mubaral Al-Kabeer Hospital, Kuwait for further investigation and treatment. A nasogastric tube was placed and about 1500 ml green-coloured fluid was drained. Since the patient developed spiking fever, he was prescribed Tazocin (piperacillin and

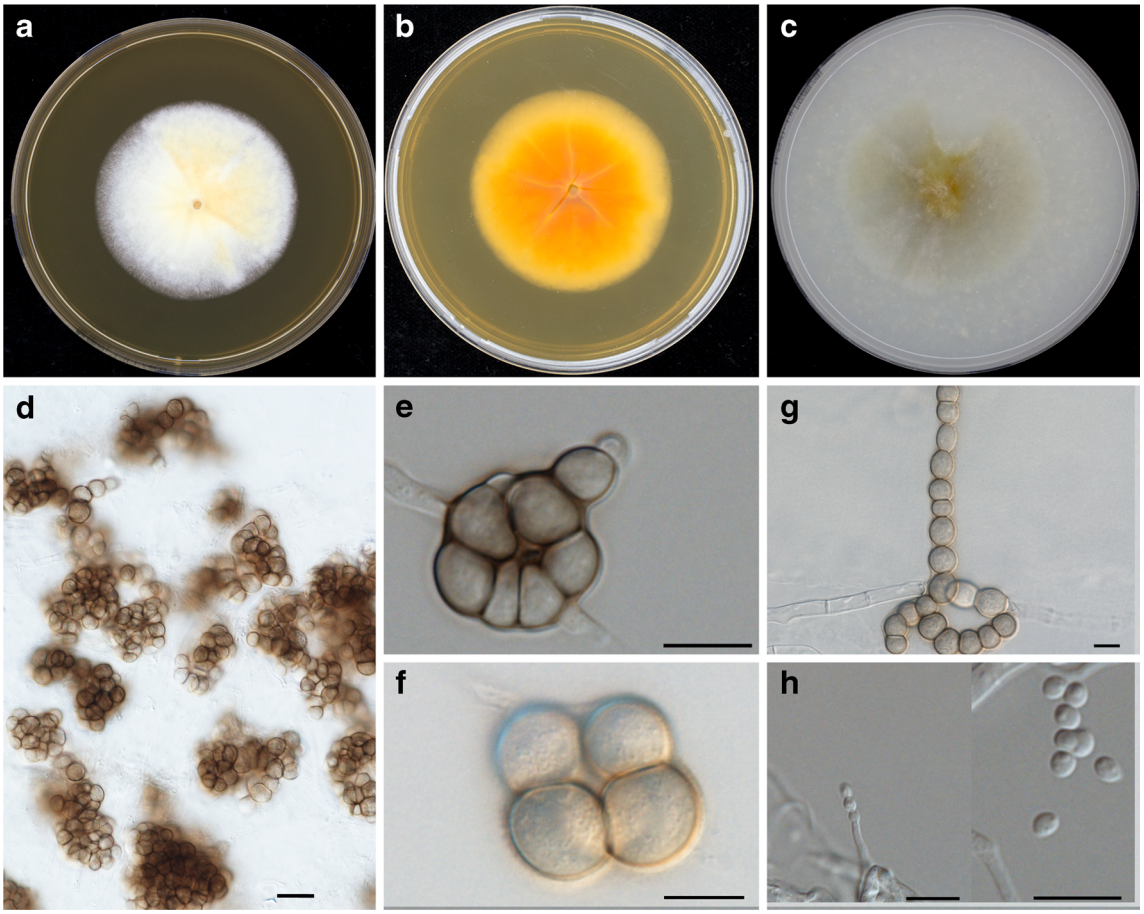


**Fig. 3** *Chaetomium anamorphosum* (CBS 137114). Colonies after 1 week of incubation on: **a**–**b** MEA obverse and reverse; **c** OA; **d**–**f** Clumps; **g** Thick-walled chlamydospore-like structures; **h** Conidiophore and conidia. — Scale bars=10 μm

tazobactam) and Flagyl (metronidazole) besides Zantac (ranitidine). Subsequently, the patient developed peritonitis with multiple pockets of fluid collection. Repeated microscopic examination of the centrifuged sediment of the peritoneal fluid, which was turbid, bloody and contained tissue-like flakes, showed septate fungal elements (Fig. 5). The culture of the peritoneal fluid yielded a fungus that grew at 37 °C. The patient was started on voriconazole 400 mg, administered twice on day one, followed by 200 mg twice daily for 15 days. He became hypotensive with signs and symptoms of septicemia. The patient was put on hemodialysis, which could not be continued due to severe hypotension. His condition deteriorated rapidly and despite therapy he died of multi-organ failure 1 week later.

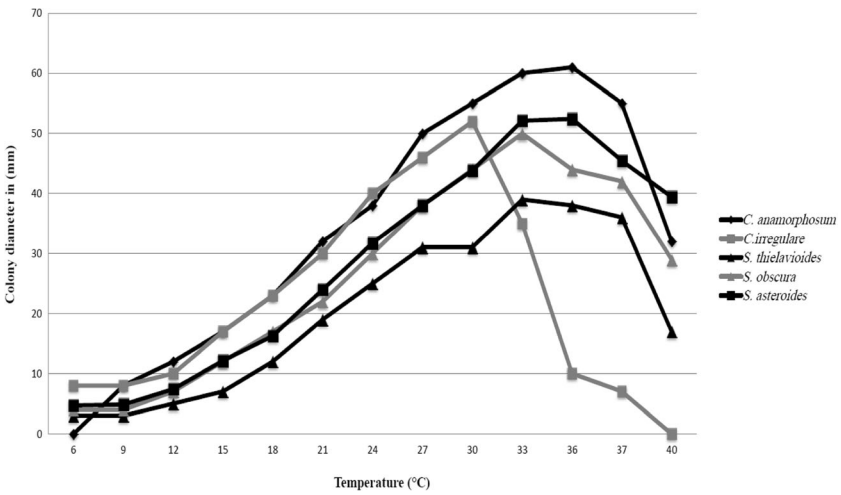


**Fig. 4** Growth rate of *Chaetomium* and *Subramaniula* species examined after 2 weeks of incubation on 2 % MEA at temperatures ranging from 6 to 40 °C

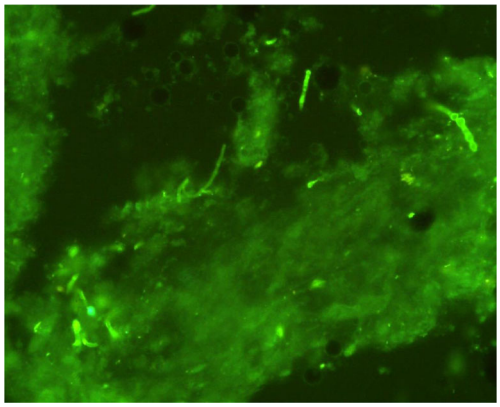

**Fig. 5** Direct fluorescence examination of peritoneal dialysis fluid from patient (CBS 137114) showing septate hyphal elements of *Chaetomium anamorphosum* in Calcofluor mount

***Subramaniula asteroides*** S.A. Ahmed, Z. U. Khan, X. Wang & de Hoog, **sp. nov.** – Fig. 6; MB 810427

Colonies on MEA radially folded, yellow green, turning dark greyish green with age; reverse dark grey. Colonies on OA felty, yellowish green or fuscous at the centre becoming faint toward the margin; some isolates showing dark, waxy colonies covered with black bulbils with age. Hyphae broad, septate, hyaline, turning dark brown with age, verruculose; part of the hyphae convert to dark brown or black, thick-walled chlamydospore-like structures. Cellular clumps irregular, black 58–100×44–71 μm, consisting of aggregates of dark brown, thick-walled cells 7–12×7–9 μm. Conidiophores phialidic, terminal or intercalary, short, hyaline, obclavate or cylindrical. Conidia hyaline 2–4×1.5–2.0 μm, unicellular, obovoidal or ellipsoidal. Minimum growth temperature 6 °C, optimum 33–36 °C, maximum above 40 °C (Fig. 4).

Holotype: dried culture in CBS Herbarium CBS H-21971; type culture CBS 123294, from corneal ulcer, V. Vinod Mootha & P. Shahinpoor, Chennai, India.

## Case reports of *Subramaniula asteroides*

(1). A 45-year-old female patient with non-insulin dependent diabetes mellitus presented to the emergency room of the Sri Ramachandra Medical College and Research Institute in Chennai, Tamil Nadu, India, with blepharospasm, photophobia and watering of the right eye. She had suffered from trauma to the right eye by a sharp

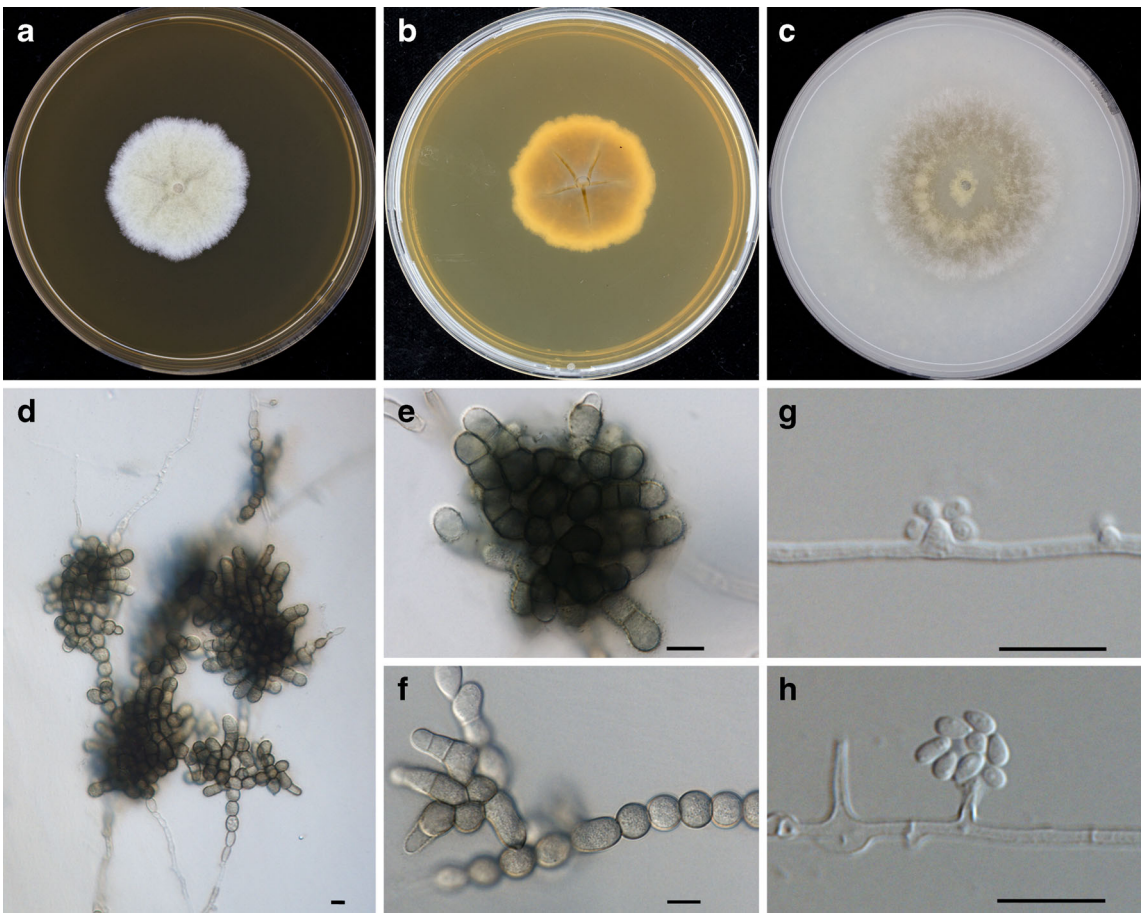


**Fig. 6** *Subramaniula asteroides* (CBS 123294). Colonies after 1 week of incubation on: **a**–**b** MEA obverse and reverse; **c** OA; **d**–**e** Clumps; **f** Thick-walled chlamydospore-like structures; **g** Conidiophores and conidia. — Scale bars=10 μm

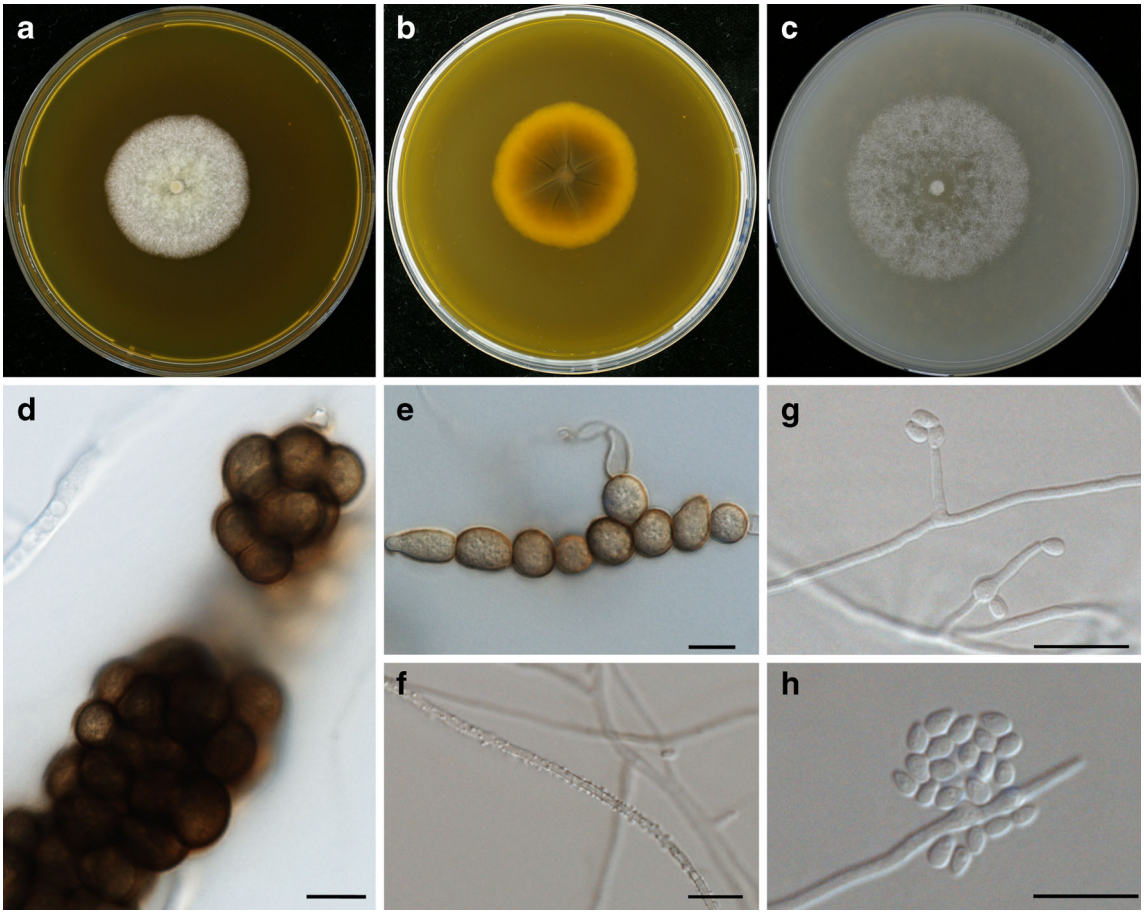


**Fig. 7** *Subramaniula obscura* (CBS 132916). Colonies after 1 week of incubation on: **a**–**b** MEA obverse and reverse; **c** OA; **d** Clumps; **e** Thick-walled chlamydospore-like structures; **f** Hyphae with wart like projections; **g**–**h** Conidiophores and conidia. — Scale bars = 10 μm

plant leaf during field work 3 days earlier. An eye examination revealed conjunctival congestion as well as a hypopyon. A corneal scraping was carried out. Direct microscopy with 10 % KOH showed septate hyphae. On Sabouraud's dextrose agar (SDA), white fungal growth was identified. The lactophenol-cotton blue mount showed hyaline hyphae with scytalidium-like arthroconidia of which few were slightly swollen. The patient was treated with amphotericin B deoxycholate 0.15 % eye drops three times daily in combination with fluconazole 200 mg p.o. twice daily for 42 days. Under this treatment, complete recovery of ocular function was obtained.

(2). A 42-year-old male patient without history of diabetes, asthma or use of steroids presented with frequent nasal block for 6 months, particularly on the right side. Symptoms were aggravated due to recurrent upper respiratory bacterial infections with staphyloccocci and *Pseudomonas* and were temporarily relieved by antibiotics such as augmentin. On local examination patient had a right-sided deviated nasal septum (DNS) with decreased fogging at this side, as demonstrated by cold spatula test. Diagnostic nasal endoscopy showed right-sided DNS with septal spur towards the right side along with blockage of the bilateral ostiomeatal complex. Ear and throat findings were normal. Patient underwent functional endoscopic sinus surgery (FESS) with septoplasty. A tissue sample was sent to the microbiology laboratory for fungal culture. Potassium hydroxide (KOH) mount showed the presence of hyphae and culture on oatmeal agar was positive.

***Subramaniula obscura*** S.A. Ahmed, Z. U. Khan, X. Wang & de Hoog, **sp. nov.** – Fig. 7, MB 810428.

Colonies on MEA floccose, greyish green; reverse dark grey. Colonies on OA felty, with fluffy, white to faint grey aerial tufts. Hyphae branched, hyaline, becoming dark brown with age, then thick-walled, verruculose with wart-like projections. Swollen hyphae with brown chlamydospore-like structures 8–13 × 8–12 μm present. Cellular clumps irregular, dark brown, 27–73 × 20–36 μm, consisting of spherical to ellipsoidal cells. Phialides erect, hyaline, cylindrical, short or long, terminal or intercalary, often remaining without conidia. Conidia unicellular, hyaline, obovoidal or clavate, 2–3 × 1.4–

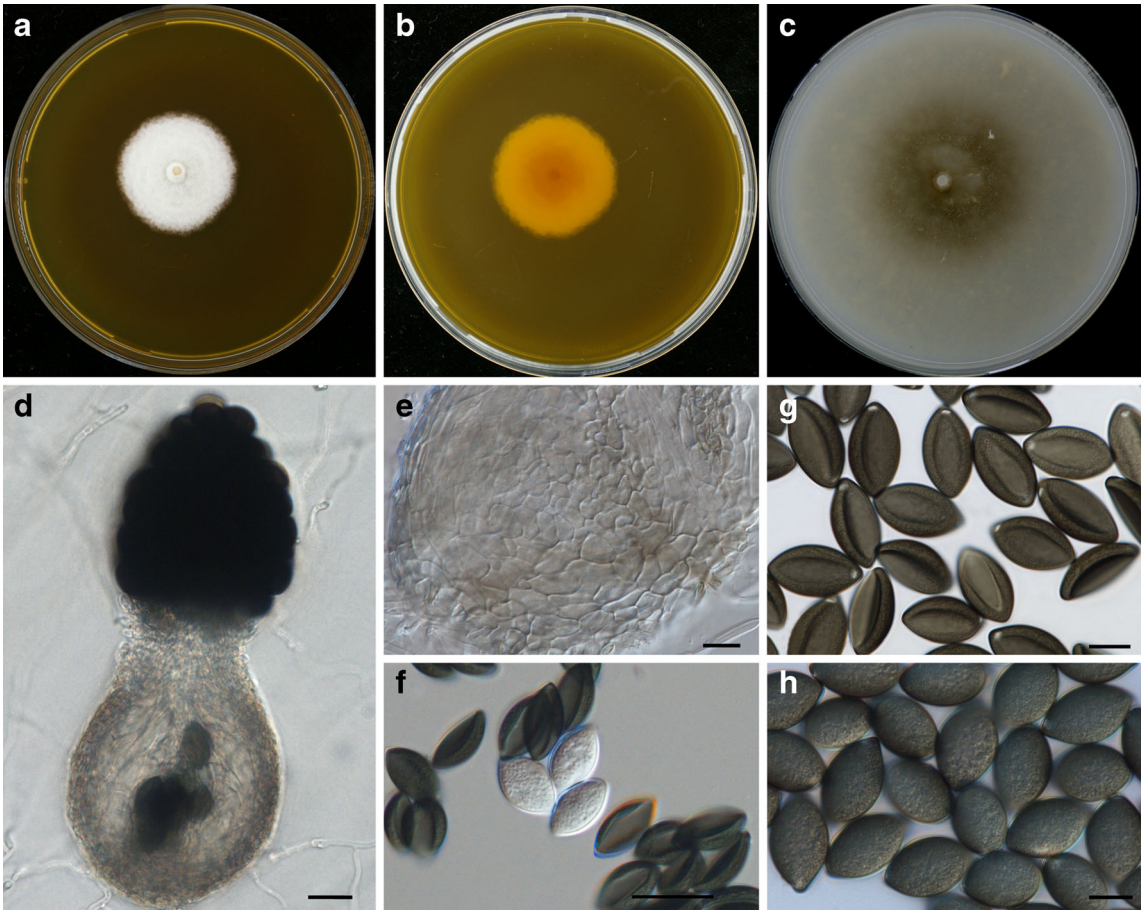


**Fig. 8** *Subramaniula thielavioides* (CBS 122.78). Colonies after 1 week of incubation on: **a**–**b** MEA obverse and reverse; **c** OA; **d** Ascomata; **e** Ascomata wall; **f**–**h** Ascospores. — Scale bars=10 μm

2.0 μm, forming pseudochains at the tips of conidiophores. Minimum growth temperature 6 °C, optimum 33 °C, maximum above 40 °C (Fig. 4).

Holotype: dried culture in CBS Herbarium, CBS H-21972; type culture CBS 123916, from male human toe infection, Kuwait, Z.U. Khan.

## Discussion

Identification of filamentous molds in the routine clinical laboratory is still mainly based on microscopic examination of sporulating colonies. Fungi that lack sporocarps or characteristic spores are difficult to identify (Pounder et al. 2007; Santos et al. 2013). In the present study 13 sterile or undiagnostic strains were analyzed. Based on phenotypic features the isolates were identified as *Papulaspora* species because of the formation of clumps or bulbil-like structures (Vinod Mootha et al. 2012). With attempts to induce sporulation using various substrates and incubation conditions, some strains produced minute conidia on short phialides, but still these conidia were not diagnostically useful and could not be used for species identification.

With molecular and phylogenetic analysis of six loci, the strains were found to belong to three new species which are described in the present study. Two species clustered with the type species of the genus *Subramaniula*, whereas one was sister to two described *Chaetomium* species. Both *Subramaniula* and *Chaetomium* are members of the family *Chaetomiaceae* (*Sordariales*). Members of this family are characterized by the formation of ascospores in deliquescent asci inside more or less extensively ornamented perithecia (Whiteside 1961). Phenotypic identification of species in *Chaetomiaceae* mostly depends on the shape of ascomatal hairs, ascoma pigmentation and ornamentation, and ascospore shape (Ames 1961; von Arx et al. 1984). The three described species in the present study exhibited unusual morphological features by failure to form sexual reproduction in ascomata. Instead, clusters of thick-walled, dark pigmented cells were present. It is unclear whether these structures represented immature perithecia (Davey et al. 2008). In the framework of singular nomenclature for fungi (Taylor 2011), the species are here nevertheless described in sexual genera on the basis of their phylogenetic position within the *Chaetomiaceae*.

The two new species clustering with *Subramaniula* originated from clinical sources, mostly eye or skin infections. The

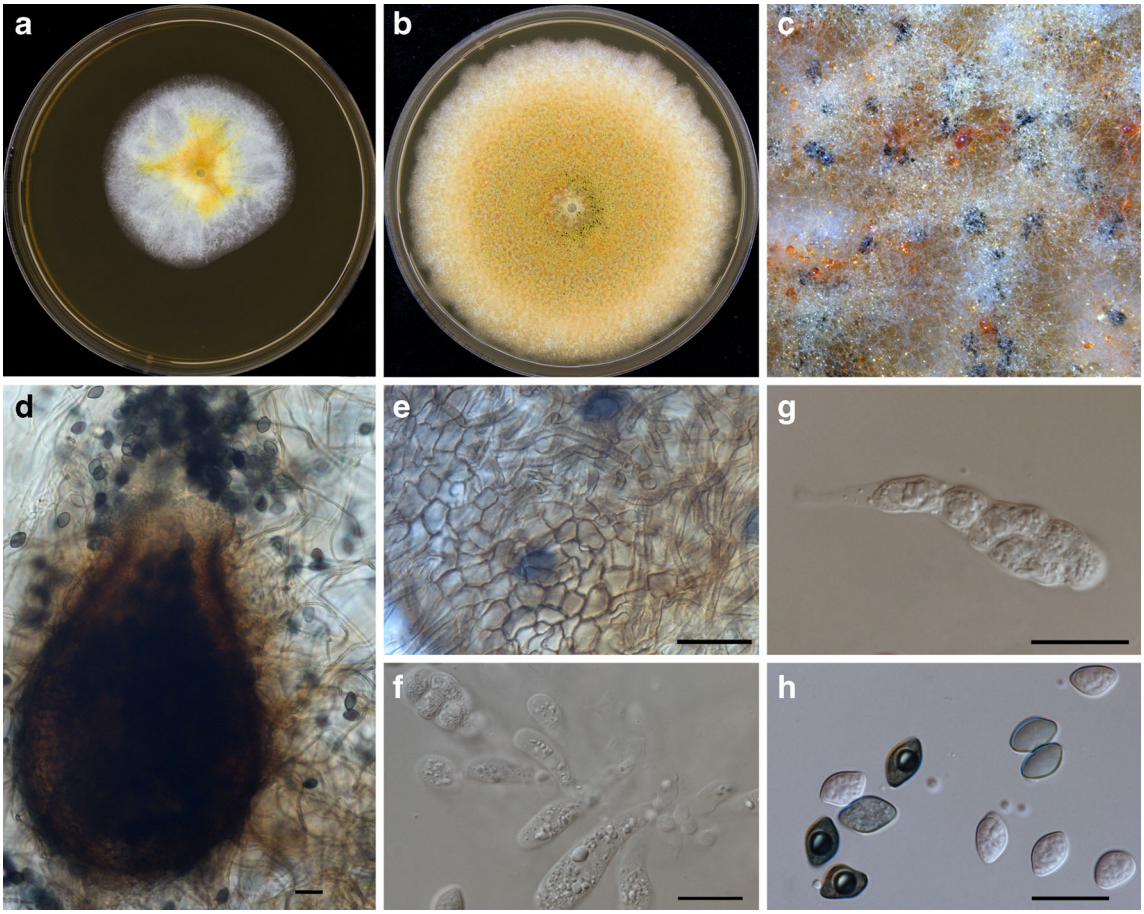


**Fig. 9** *Chaetomium irregulare* (CBS 446.66). Colonies on MEA: **a** One week **b** Four weeks of incubation; **c** Asci on the surface of OA agar; **d** Ascomata; **e** Ascomata wall; **f**–**g** Asci; h. Ascospores. — Scale bars=10 μm

coprophilous genus *Subramaniula* is presently known from two species that were transferred from *Achaetomium*, i.e. *S. irregularis* and the generic type species, *S. thielavioides* (Cannon 1986; von Arx 1985; von Arx et al. 1978). The genus *Subramaniula* is characterized by glabrous pale ascomata with wide apical openings (Fig. 8), without a known asexual morph. Cannon (1986) reported "A few hyphae with chains of considerably swollen cells, producing thin-walled globose or ellipsoidal bodies to 8 μm diam, but may well not function as propagule". Some authors suggested that these bodies might be related to the asexual state of this fungus (Pastirčák and Pastirčáková 2009). Upon the examination of the ex-type strain of *S. thielavioides* (CBS 122.78), we detected chains of swollen cells, but no thin-walled bodies were seen. Compared with our two novel *Subramaniula* species in which conidia were observed, these structures are unlikely to be the conidia, but may act as survival propagules. *Subramaniula irregularis* (syn. *Achaetomiella irregulare*) is described as morphologically similar to *S. thielavioides* with some differences in shape and size of ascospores and pigmentation of ascomata but no living culture is available for this species (Cannon 1986).

*Subramaniula thielavioides* has been isolated from a dung sample from India, whereas *S. irregularis* is only known from soil in South Africa (Cannon 1986; von Arx et al. 1978). Thus far no human infection has been sttributed to *Subramaniula*. However, Cannon (1986) reported the isolation of *S. thielavioides* from a human nail and interestingly he reported "Medical mycologists should be aware of its existence". With the two newly described clinical species, the genus *Subramaniula* is confirmed to have a potential as an opportunistic genus eventually causing skin, eye, and nail infections. An unidentified strain from a corneal ulcer published by Vinod Mootha et al. (2012), CBS 123294, is described in the present study as a novel species *Subramaniula asteroides*. The strain was recovered from a patient wearing a contact lens and who was injured with a wire while working in a horse stable. Strain dH 21571 concerned a keratitis emerging after eye trauma with a sharp plant leaf on an agricultural field in tropical India. Two further strains from the U.S.A. were also derived from corneal ulcers. Two more strains from eye infections in Spain matched with *S. asteroides*; CNM-CM 4314 was isolated from corneal exudate of a 27-year-old male patient, CNM-CM 7482 originated from an endophthalmitis of a

47-year-old immunosuppressed patient; hyphyae were observed in the vitreous humour and the patient lost the eye. *Subramaniula asteroides* thus seems to have a strong association with traumatic eye infections. During the course of this study another sequence derived from an isolate (UTHSC 03-1315) from skin of a patient from Saudi Arabia was also identified as *S. asteroides*. The only environmental isolate of *S. asteroides* also originated from Saudi Arabia, namely from sandy desert soil under hydrocarbon impact. It was recovered by toluene enrichment (Zhao et al. 2010), a method designed for the isolation of fungi growing under toxic conditions.

*Subramaniula obscura* is described in the present paper for a single strain from a 53-year-old Kuwaiti male present with a toe infection. The strain was initially identified as *Chaetomium cuniculorum*. We found that strains of *C. cuniculorum* are phylogenetically related to *S. obscura*, as both were found in a single, supported clade based on both ribosomal gene analysis (ML-BS/PP, 84 %/1.0) and multilocus analysis of protein coding genes (ML-BS/PP, 100 %/0.99) (Fig. 2). With ITS sequencing and using a limited number of taxa, separation of the two species might indeed be difficult. The ITS sequence of the ex-type strain of *S. obscura*, CBS 132916, showed 97 % similarity to *C. cuniculorum* isolates. The latter species is thought to occur mostly on dung and about half of the *C. cuniculorum* strains maintained in the CBS culture collection originated from dung of herbivorous animals. ITS similarity of *S. obscura* to *S. thielavioides* is 99.4 %, but protein coding genes are consistently different and therefore description of the novel species in *Subramaniula* is warranted.

The macro- and micro-morphology of *Subramaniula obscura* is similar to that of *S. asteroides* except in that the cells forming the clumps of *S. obscura* are more brownish and rounded, and hyphae are thicker and with wart-like protrusions. The minimum growth temperature for both species and for *S. thielavioides* is 6 °C, and they all grew very well above 40 °C indicating thermotolerance of *Subramaniula* species. Optimum growth was between 33 and 36 °C, and consistent survival at 37 °C denotes the ability to grow at human body temperature as virulence factor (Revankar and Sutton 2010).

Identification of *Chaetomium anamorphosum* strain CBS 137114 as a novel species, recovered from human peritonitis, was initially done by morphological comparison with described *Papulaspora* species. This strain showed some similarity to *Papulaspora nishigaharanas* by formation of aggregates of brown-coloured, thick-walled cells, as well as by production of phialoconidia (Watanabe 1991). Comparison of sequences of ITS and of the D1/D2 region of 28S rRNA gene in GenBank did not reveal homology with any known sequenced species. With the molecular phylogenetic approach we were able to resolve the taxonomy of this isolate as a close relative of the ascosporulating species *Chaetomium irregulare* and *C. fusisporum* (Figs. 1, 2, and 9). The newly identified species differed from *C. irregulare* and *C. fusisporum*, not only in morphology but also in the growth temperature. The optimum growth temperature of *C. anamorphosum* was 33–36 °C and growth was still observed at 40 °C, whereas for *C. irregulare* the optimum was 30 °C and it was unable to grow or grew only poorly at 40 °C. *Chaetomium irregulare* was transferred to the genus *Achaetomium* by Rodríguez et al. (2004). However, when compared with the generic type *Achaetomium globosum*, it was found to be distant and the name *Achaetomium irregulare* is redundant. The taxonomy and nomenclature of the clade containing the new species and other *Chaetomium* spp. as paraphyletic to *Subramaniula* are unclear because of the relatively large distance to the generic type species, *C. globosum*. In addition, the morphology of ascomata, ascomatal hairs if present, and ascospores of the species of this clade differs from that of *C. globosum*. The genera *Chaetomium*, *Achaetomium*, *Subramaniula*, and *Achaetomiella* were morphologically similar with only few differences in the presence and absence of ascomatal hairs, ascoma wall, ascospore color, and in growth rate (Cannon 1986). These characters have become largely irrelevant due to the availability of molecular data. Pending a taxonomic revision of *Chaetomium*, we maintain the current classification of the cluster within this genus, acknowledging *Subramaniula* as a genus nested within *Chaetomium*.

Despite the large number of described species in *Chaetomium* and relatives, little is known about their molecular taxonomy and only few species have been sequenced and are available in public databases (Wang et al. 2014). Most of the previous studies were restricted to rare species, and the phylogeny of more ubiquitous *Chaetomium* species remains unresolved (Asgari and Zare 2011; Lee and Hanlin 1999; Wang et al. 2014). Recently, three new species in the *C. indicum* group were described based on molecular analysis of four genes (Wang et al. 2014). Another molecular study of *Chaetomium* was that of Asgari and Zare (2011) in Iran using phylogenetic analysis of three genes resulting in the delimitation of six new species. Two of these showed asexual morphs similar to those formed by *Chaetomium anamorphosum* and by both *Subramaniula* species described in the present paper. *Chaetomium rectangulare* Asgari and Zare (2011) belongs to the *C. globosum* group characterized by asexual morphs with phialides and conidia closely similar to those of our new species. This might indicate that the new species somehow lost their ability to form sexual fruiting and replaced this by an asexual type of sporulation. Sequencing of the *MAT* locus regulating sexual reproduction might indicate whether the sexual morphs in these species are absent or suppressed.

Animal dung, decaying vegetable matter and soil are known natural habitats for *Chaetomium*, *Subramaniula* and *Papulaspora* species. De Hoog et al. (2013b) noticed that the genus *Madurella* is nested within *Chaetomium* and that many species in the *Chaetomiaceae* combine dung-

association with arid climatic conditions. Adding the previously unidentified non-ascosporulating species to *Chaetomium*, the role of this genus in human and animal disease has increased significantly. *Chaetomium* infections and infections by species clustering in the *Chaetomium* phylogenetic tree, such as *Chaetomidium* and *Thielavia*, have been reported from skin, hair, and nails (Hubka et al. 2011; Kaliamurthy et al. 2011; Najafzadeh et al. 2014; Vinod Mootha et al. 2012). Moreover, several species of the *Chaetomiaceae* have been reported to cause serious opportunistic infections in immunocompromised patients (Al-Aidaroos et al. 2007; Guppy et al. 1998; Hoppin et al. 1983). Several cases of peritonitis similar to ours in *C. anamorphosum* have been reported in immunocompromised individuals who had acquired the infection during peritoneal dialysis. Febré et al. (1999) isolated *C. globosum* from bottles of dialysis fluid; peritonitis was reported by Baer et al. (2013). An interesting case published by Issa et al. (2013) concerned a peritonitis in an immunocompetent female from Damam, Saudi Arabia, caused by an unknown *Chaetomium* species. Apparently also immunocompetent individuals are at risk of deep infections by *Chaetomium* or related fungi. As an example, a case of fatal cerebral phaeohyphomycosis caused by *Thielavia subthermophila* was reported in immunocompetent individuals (Badali et al. 2011). *Chaetomium atrobrunneum*, *C. perlucidum*, and *C. strumarium* were now regarded as neurotropic species causing serious and life-threatening infections (Abbott et al. 1995; Barron et al. 2003; Guppy et al. 1998). It seems that members of *Chaetomiaceae* indeed have an underestimated clinical potential, and re-evaluation of the role of the genus in human pathology is urgently required. The natural habitat of many species in arid climates and their survival of high temperatures probably enhance their survival in mammalian tissue. The ability of *C. anamorphosum* to grow optimally at 36 °C, in contrast to *C. irregulare*, is a good example. The species are able to break the thermal exclusionary zone of the human body and if there is immunosuppression or other immunological problem fungi can emerge as potential human pathogens (Casadevall 2012).

Due to identification difficulties on the basis of phenotypic criteria, some older cases of *Chaetomium* or *Subramaniula* species might have erroneously been disregarded or reported as cases of *Madurella* or *Papulaspora* infection (Mohd-Tahir et al. 2012). Our studies clearly show that traumatic and opportunistic infections by chaetomium-like species often yield non- or poorly-sporulating strains in culture (Najafzadeh et al. 2014; Vinod Mootha et al. 2012). Description of these strains as separate taxonomic entities in the genus is significant from both clinical and epidemiological points of view. Moreover, antifungal susceptibility studies are scant, and treatment protocols are urgently needed.

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